

09/325.189

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NEWS 1 Web Page URLs for STN Seminar Schedule - N. America
NEWS 2 Dec 17 The CA Lexicon available in the CAPLUS and CA files
NEWS 3 Feb 06 Engineering Information Encompass files have new names
NEWS 4 Feb 16 TOXLINE no longer being updated
NEWS 5 Apr 23 Search Derwent WPINDEX by chemical structure
NEWS 6 Apr 23 PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
NEWS 7 May 07 DGENE Reload
NEWS 8 Jun 20 Published patent applications (A1) are now in USPATFULL
NEWS 9 JUL 13 New SDI alert frequency now available in Derwent's
DWPI and DPCI
NEWS 10 Aug 23 In-process records and more frequent updates now in
MEDLINE
NEWS 11 Aug 23 PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
NEWS 12 Aug 23 Adis Newsletters (ADISNEWS) now available on STN
NEWS 13 Sep 17 IMSworld Pharmaceutical Company Directory name change
to PHARMASEARCH
NEWS 14 Oct 09 Korean abstracts now included in Derwent World Patents
Index
NEWS 15 Oct 09 Number of Derwent World Patents Index updates increased
NEWS 16 Oct 15 Calculated properties now in the REGISTRY/ZREGISTRY File
NEWS 17 Oct 22 Over 1 million reactions added to CASREACT
NEWS 18 Oct 22 DGENE GETSIM has been improved
NEWS 19 Oct 29 AAASD no longer available
NEWS 20 Nov 19 New Search Capabilities USPATFULL and USPAT2
NEWS 21 Nov 19 TOXCENTER(SM) - new toxicology file now available on STN

NEWS EXPRESS August 15 CURRENT WINDOWS VERSION IS V6.0c,
CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP),
AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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FILE 'MEDLINE' ENTERED AT 11:49:10 ON 24 NOV 2001

=> s fluorescence correlation spectroscop? (10a)(nucleic acid amplif? or PCR) (10a)
quantif?

L1 1 FLUORESCENCE CORRELATION SPECTROSCOP? (10A) (NUCLEIC ACID AMPLIF?
OR PCR) (10A) QUANTIF?

=> d l1 bib ab

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS

AN 1997:470302 CAPLUS

DN 127:76986

TI Process for determination of nucleic acid molecules at low concentrations
by amplification using labeled primers

IN Eigen, Manfred; Walter, Nils; Schwille, Petra; Oehlenschlager, Frank

PA Evotec Biosystems GmbH, Germany; Eigen, Manfred; Walter, Nils; Schwille,
Petra; Oehlenschlager, Frank

SO PCT Int. Appl., 41 pp.

CODEN: PIXXD2

DT Patent

LA German

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9721832	A1	19970619	WO 1996-EP5472	19961206

W: JP, US

RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

DE 19545965	A1	19970612	DE 1995-19545965	19951208
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DE 19634226	A1	19980226	DE 1996-19634226	19960824
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PRAI	DE 1995-19545965	19951208
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	DE 1996-19634226	19960824
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AB A method for detn. of a low concn. of nucleic acid mols. in samples to be
examd., by amplification reactions using a combination of unmarked primers
as amplification primers and detectable, in particular labeled primers, as
detection primers and in particular using polymerases and/or ligases. The
detectable primers are incorporated into the amplification products. The
drop in the concn. of the free labeled primers, or the rise in the concn.
of amplification products is measured and is used to det. the no. of mols.
of target sequence in the original sample. Incorporation of fluorescent
group-labeled primers can be quantified by fluorescence correlation
spectrometry. The method is insensitive to non-specific amplification.

=> d l1 kwic

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS

ST nucleic acid detection amplification labeled primer; **fluorescence**
correlation spectroscopy PCR DNA
quantification

```
=> s l1 and (nucleotides(10a)label?)
L2          0 L1 AND (NUCLEOTIDES(10A) LABEL?)

=> s l1 and nucleotide#
L3          0 L1 AND NUCLEOTIDE#

=> s (nucleotide#(10a)label##) (10a) (substrate#(10a)label##)
L4          118 (NUCLEOTIDE#(10A) LABEL##) (10A) (SUBSTRATE#(10A) LABEL##)

=> s l4 and fluorescence correlation spectroscop?
L5          0 L4 AND FLUORESCENCE CORRELATION SPECTROSCOP?

=> s (nucleotide#(10a)label##) or (substrate#(10a)label##)
L6          16834 (NUCLEOTIDE#(10A) LABEL##) OR (SUBSTRATE#(10A) LABEL##)

=> s l6 and fluorescen? correlation spectroscop?
L7          17 L6 AND FLUORESCEN? CORRELATION SPECTROSCOP?

=> s l7 and ((nucleic acid amplif?) or PCR))
UNMATCHED RIGHT PARENTHESIS 'PCR))'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s l7 and ((nucleic acid amplif?) or PCR)
L8          4 L7 AND ((NUCLEIC ACID AMPLIF?) OR PCR)

=> d l8 bib ab kwic
```

```
L8  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2001 ACS
AN  2001:731083  CAPLUS
DN  135:269641
TI  Single nucleotide polymorphism detection via
    fluorescence correlation spectroscopy analysis
    of labeled probe hybridization
IN  Hori, Kunio; Karaki, Sachiko; Kanou, Tokio; Takamiya, Yuji
PA  Olympus Optical Co., Ltd., Japan
SO  PCT Int. Appl., 63 pp.
    CODEN: PIXXD2
DT  Patent
LA  Japanese
FAN.CNT 3
```

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
PI	WO 2001073120	A1	20011004	WO 2001-JP2495	20010327
	W: US				
	RW: DE, FR, GB				
	JP 2001269198	A2	20011002	JP 2000-87500	20000327
	JP 2001269199	A2	20011002	JP 2000-87501	20000327
	JP 2001272404	A2	20011005	JP 2000-87504	20000327
PRAI	JP 2000-87500	A	20000327		
	JP 2000-87501	A	20000327		
	JP 2000-87504	A	20000327		

```
AB  Methods for detection of single nucleotide polymorphism (SNP)
    via hybridization of sequence-specific labeled probes and
    fluorescence correlation spectroscopy (FCS)
    anal., is disclosed. Fluorescence signal is detected by optical
    measurement of fluorescent probes movement as function of time during
    hybridization. The method allows simple detection of SNP without the need
    for B/F sepn., PCR or electrophoresis.
```

```
RE.CNT 8
```

```
RE
(1) Collaborative Res Inc; WO 9012115 A 1990 CAPLUS
(3) Evotec Biosystems Gmbh; DE 4301005 A CAPLUS
(4) Evotec Biosystems Gmbh; EP 672951 A CAPLUS
```

(6) Kinjo, M; Bio Techniques 1998, V25(4), P706 CAPLUS
 (7) Olympus Optical Company Limited; DE 19950823 A1 CAPLUS
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy analysis
 of **labeled** probe hybridization

AB Methods for detection of single **nucleotide** polymorphism (SNP)
 via hybridization of sequence-specific **labeled** probes and
fluorescence correlation spectroscopy (FCS)
 anal., is disclosed. Fluorescence signal is detected by optical
 measurement of fluorescent probes movement as function of time during
 hybridization. The method allows simple detection of SNP without the need
 for B/F sepn., PCR or electrophoresis.

ST genotyping polymorphism **labeled** probe hybridization; single nucleotide
 polymorphism **fluorescence correlation**
spectroscopy probe hybridization

IT Gene, animal
 RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical
 study); BIOL (Biological study)
 (HLA, SNP anal. of; single **nucleotide** polymorphism detection
 via **fluorescence correlation spectroscopy**
 anal. of **labeled** probe hybridization)

IT Histocompatibility antigens
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (SNP anal. of; single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy anal.
 of **labeled** probe hybridization)

IT Microscopes
 (confocal,; single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy anal.
 of **labeled** probe hybridization)

IT Fluorometry
 (correlation; single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy anal.
 of **labeled** probe hybridization)

IT Oligonucleotides
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST
 (Analytical study); BIOL (Biological study); USES (Uses)
 (**labeled**; single **nucleotide** polymorphism detection
 via **fluorescence correlation spectroscopy**
 anal. of **labeled** probe hybridization)

IT Fluorescent probes
 Genetic polymorphism
 Genotyping (method)
 Nucleic acid hybridization
 (single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy anal.
 of **labeled** probe hybridization)

IT Probes (nucleic acid)
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST
 (Analytical study); BIOL (Biological study); USES (Uses)
 (single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy anal.
 of **labeled** probe hybridization)

IT Genetic polymorphism
 (single **nucleotide**; single **nucleotide** polymorphism
 detection via **fluorescence correlation**
spectroscopy anal. of **labeled** probe hybridization)

IT Antigens
 RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (surface, red blood cell; single **nucleotide** polymorphism
 detection via **fluorescence correlation**
spectroscopy anal. of **labeled** probe hybridization)

IT 37228-74-3, Exonuclease

RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL
(Biological study); USES (Uses)
(use in genotyping; single **nucleotide** polymorphism detection
via **fluorescence correlation spectroscopy**
anal. of **labeled** probe hybridization)

=> d 18 1-4 bib ab kwic

L8 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS

AN 2001:731083 CAPLUS

DN 135:269641

TI Single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy analysis
of **labeled** probe hybridization

IN Hori, Kunio; Karaki, Sachiko; Kanou, Tokio; Takamiya, Yuji

PA Olympus Optical Co., Ltd., Japan

SO PCT Int. Appl., 63 pp.

CODEN: PIXXD2

DT Patent

LA Japanese

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001073120	A1	20011004	WO 2001-JP2495	20010327
	W: US				
	RW: DE, FR, GB				
	JP 2001269198	A2	20011002	JP 2000-87500	20000327
	JP 2001269199	A2	20011002	JP 2000-87501	20000327
	JP 2001272404	A2	20011005	JP 2000-87504	20000327
PRAI	JP 2000-87500	A	20000327		
	JP 2000-87501	A	20000327		
	JP 2000-87504	A	20000327		

AB Methods for detection of single **nucleotide** polymorphism (SNP)
via hybridization of sequence-specific **labeled** probes and
fluorescence correlation spectroscopy (FCS)
anal., is disclosed. Fluorescence signal is detected by optical
measurement of fluorescent probes movement as function of time during
hybridization. The method allows simple detection of SNP without the need
for B/F sepn., **PCR** or electrophoresis.

RE.CNT 8

RE

- (1) Collaborative Res Inc; WO 9012115 A 1990 CAPLUS
- (3) Evotec Biosystems Gmbh; DE 4301005 A CAPLUS
- (4) Evotec Biosystems Gmbh; EP 672951 A CAPLUS
- (6) Kinjo, M; Bio Techniques 1998, V25(4), P706 CAPLUS
- (7) Olympus Optical Company Limited; DE 19950823 A1 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Single **nucleotide** polymorphism detection via
fluorescence correlation spectroscopy analysis
of **labeled** probe hybridization

AB Methods for detection of single **nucleotide** polymorphism (SNP)
via hybridization of sequence-specific **labeled** probes and
fluorescence correlation spectroscopy (FCS)
anal., is disclosed. Fluorescence signal is detected by optical
measurement of fluorescent probes movement as function of time during
hybridization. The method allows simple detection of SNP without the need
for B/F sepn., **PCR** or electrophoresis.

ST genotyping polymorphism **labeled** probe hybridization; single **nucleotide**
polymorphism **fluorescence correlation**
spectroscopy probe hybridization

IT Gene, animal

RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical
study); BIOL (Biological study)

(HLA, SNP anal. of; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Histocompatibility antigens
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (SNP anal. of; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Microscopes
 (confocal,; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Fluorometry
 (correlation; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Oligonucleotides
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses) (**labeled**; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Fluorescent probes
 Genetic polymorphism
 Genotyping (method)
 Nucleic acid hybridization
 (single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Probes (nucleic acid)
 RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses) (single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Genetic polymorphism
 (single **nucleotide**; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT Antigens
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (surface, red blood cell; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

IT 37228-74-3, Exonuclease
 RL: BUU (Biological use, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses) (use in genotyping; single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** anal. of **labeled** probe hybridization)

L8 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS
 AN 2001:717144 CAPLUS
 DN 135:269638
 TI Single **nucleotide** polymorphism detection via **fluorescence correlation spectroscopy** analysis of **labeled** probe hybridization
 IN Hori, Kunio
 PA Olympus Optical Co., Ltd., Japan
 SO Jpn. Kokai Tokkyo Koho, 7 pp.
 CODEN: JKXXAF
 DT Patent
 LA Japanese
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2001269199	A2	20011002	JP 2000-87501	20000327
	WO 2001073120	A1	20011004	WO 2001-JP2495	20010327
	W: US				
	RW: DE, FR, GB				
PRAI	JP 2000-87500	A	20000327		
	JP 2000-87501	A	20000327		
	JP 2000-87504	A	20000327		
AB	Methods for detection of single nucleotide polymorphism (SNP) via hybridization of sequence-specific labeled probes and fluorescence correlation spectroscopy (FCS) anal., is disclosed. Fluorescence signal is detected by optical measurement of fluorescent probes movement as function of time during hybridization. Brownian motion is detected by FCS. The method allows simple detection of SNP without the need for B/F sepn., PCR or electrophoresis.				
TI	Single nucleotide polymorphism detection via fluorescence correlation spectroscopy analysis of labeled probe hybridization				
AB	Methods for detection of single nucleotide polymorphism (SNP) via hybridization of sequence-specific labeled probes and fluorescence correlation spectroscopy (FCS) anal., is disclosed. Fluorescence signal is detected by optical measurement of fluorescent probes movement as function of time during hybridization. Brownian motion is detected by FCS. The method allows simple detection of SNP without the need for B/F sepn., PCR or electrophoresis.				
ST	single nucleotide polymorphism fluorescence correlation spectroscopy probe hybridization				
IT	Fluorometry (correlation; single nucleotide polymorphism detection via fluorescence correlation spectroscopy anal. of labeled probe hybridization)				
IT	Brownian motion (of fluorescent probes, FCS detection; single nucleotide polymorphism detection via fluorescence correlation spectroscopy anal. of labeled probe hybridization)				
IT	Fluorescent probes Nucleic acid hybridization (single nucleotide polymorphism detection via fluorescence correlation spectroscopy anal. of labeled probe hybridization)				
IT	Probes (nucleic acid) RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses) (single nucleotide polymorphism detection via fluorescence correlation spectroscopy anal. of labeled probe hybridization)				
IT	Genetic polymorphism (single nucleotide ; single nucleotide polymorphism detection via fluorescence correlation spectroscopy anal. of labeled probe hybridization)				
L8	ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS				
AN	2000:408542 CAPLUS				
DN	133:40224				
TI	Method and the apparatus for quantitative analysis of the target nucleic acid by polymerase chain reaction and fluorescence correlation spectroscopy				
IN	Kinjo, Masataka				
PA	Japan				
SO	Jpn. Kokai Tokkyo Koho, 11 pp. CODEN: JKXXAF				

DT Patent
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 2000166598	A2	20000620	JP 1998-347493	19981207
AB	A method and app. for quant. anal. of the target nucleic acid by polymerase chain reaction (PCR) and fluorescence correlation spectroscopy (FCS) is disclosed. Nucleic acid amplification is carried out in the presence of primers, labeled substrate , DNA polymerase, and the target nucleic acid. The method also comprises the steps of measurement of signals from the label, assessment of the soln. mobility of the label, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The app. includes a microscope. The method was applied to the quant. anal. of lambda phage DNA using a set of primers and fluorescein-11-dUTP. DNA amplification by the polymerase chain reaction (PCR) was monitored at the level of single mols. The technique used consisted of a direct fluorescent labeling method using the PCR and measurement of fluorescence fluctuation by fluorescence correlation spectroscopy (FCS). An increasing no. of target DNA mols. during amplification resulted in a decrease of the no. fluctuations, and also an increase of the av. diffusion time. Fluorescein-11-dUTP was incorporated into the DNA strand with a length of 4000 bp using Taq DNA polymerase. Increasing the apparent labeling d. according to concn. of fluorescein-11-dUTP was evaluated from the fluorescence intensity per DNA mol. The no. of amplified DNA mols. could be detected quant. after 10 PCR cycles even when the initial template no. was 3750 copies; however, a linear relationship between the initial template no. and amplified DNA no. was shown at 20 cycles in PCR.				
TI	Method and the apparatus for quantitative analysis of the target nucleic acid by polymerase chain reaction and fluorescence correlation spectroscopy				
AB	A method and app. for quant. anal. of the target nucleic acid by polymerase chain reaction (PCR) and fluorescence correlation spectroscopy (FCS) is disclosed. Nucleic acid amplification is carried out in the presence of primers, labeled substrate , DNA polymerase, and the target nucleic acid. The method also comprises the steps of measurement of signals from the label, assessment of the soln. mobility of the label, and quantification of the target nucleic acid. Statistical anal. of the data by performing arithmetic operations using the autocorrelation function is also included. The app. includes a microscope. The method was applied to the quant. anal. of lambda phage DNA using a set of primers and fluorescein-11-dUTP. DNA amplification by the polymerase chain reaction (PCR) was monitored at the level of single mols. The technique used consisted of a direct fluorescent labeling method using the PCR and measurement of fluorescence fluctuation by fluorescence correlation spectroscopy (FCS). An increasing no. of target DNA mols. during amplification resulted in a decrease of the no. fluctuations, and also an increase of the av. diffusion time. Fluorescein-11-dUTP was incorporated into the DNA strand with a length of 4000 bp using Taq DNA polymerase. Increasing the apparent labeling d. according to concn. of fluorescein-11-dUTP was evaluated from the fluorescence intensity per DNA mol. The no. of amplified DNA mols. could be detected quant. after 10 PCR cycles even when the initial template no. was 3750 copies; however, a linear relationship between the initial template no. and amplified DNA no. was shown at 20 cycles in PCR.				
ST	app nucleic acid quantification arithmetic statistics microscope; DNA amplification PCR fluorescence correlation spectroscopy				

- IT Analytical apparatus
(biochem.; method and app. for quant. anal. of target nucleic acid by
polymerase chain reaction and **fluorescence
correlation spectroscopy**)
- IT Fluorometry
(correlation, (FCS); method and app. for quant. anal. of target nucleic
acid by polymerase chain reaction and **fluorescence
correlation spectroscopy**)
- IT **Nucleotides**, biological studies
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST
(Analytical study); BIOL (Biological study); USES (Uses)
(**labeled**; method and app. for quant. anal. of target nucleic
acid by polymerase chain reaction and **fluorescence
correlation spectroscopy**)
- IT Algorithm
Autocorrelation function
Fluorescent indicators
Microscopes
Nucleic acid amplification (method)
PCR (polymerase chain reaction)
Statistical analysis
(method and app. for quant. anal. of target nucleic acid by polymerase
chain reaction and **fluorescence correlation
spectroscopy**)
- IT Nucleic acids
RL: ANT (Analyte); ANST (Analytical study)
(method and app. for quant. anal. of target nucleic acid by polymerase
chain reaction and **fluorescence correlation
spectroscopy**)
- IT Primers (nucleic acid)
RL: BPR (Biological process); BUU (Biological use, unclassified); BIOL
(Biological study); PROC (Process); USES (Uses)
(method and app. for quant. anal. of target nucleic acid by polymerase
chain reaction and **fluorescence correlation
spectroscopy**)
- IT Diffusion
(of **labeled substrate** in soln., measurement of;
method and app. for quant. anal. of target nucleic acid by polymerase
chain reaction and **fluorescence correlation
spectroscopy**)
- IT 134344-32-4, Fluorescein-11-dUTP
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST
(Analytical study); BIOL (Biological study); USES (Uses)
(**labeled substrate**; method and app. for quant.
anal. of target nucleic acid by polymerase chain reaction and
fluorescence correlation spectroscopy)
- IT 9012-90-2, DNA polymerase
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
(Uses)
(method and app. for quant. anal. of target nucleic acid by polymerase
chain reaction and **fluorescence correlation
spectroscopy**)

L8 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2001 ACS

AN 1996:607588 CAPLUS

DN 125:240228

TI Detection of nucleic acid sequences at very low concentrations using
multiple probes with fluorescent labels

IN Eigen, Manfred Prof; Rigler, Rudolf

PA Max-Planck-Gesellschaft Zur Foerderung Der Wissenschaften E.V. Berlin,
Germany

SO Eur. Pat. Appl., 14 pp.

CODEN: EPXXDW

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 731173	A2	19960911	EP 1995-119546	19951212
	EP 731173	A3	20000223		
	R: CH, DE, FR, GB, IT, LI				
	DE 19508366	A1	19960912	DE 1995-19508366	19950310
	DE 19508366	C2	19980129		
	JP 08242858	A2	19960924	JP 1995-348201	19951219
	US 5807677	A	19980915	US 1995-574916	19951219
PRAI	DE 1995-19508366		19950310		

AB A method of detecting a specific nucleic acid sequence at very low levels using multiple hybridization probes labeled with a fluorescent reporter group is described. The target is hybridized with a mixt. of labeled probes and excess probe is removed after hybridization, particularly electrophoretically, esp. using a very high frequency a.c. (.gtoreq.100 kHz). This generates an electrophoretic trap that rapidly removes the probes from the origin, leaving hybridized probes and the target sequence near the origin where they can be quantified by **fluorescence correlation spectroscopy**. Peptide nucleic acids carrying a neutralizing charge may be used as probes. This lowers the mobility of the hybrid even farther compared to the probes themselves. The method is intended for use on very small (femtoliter) vols. and can be used to detect the amplification products of single copies of a sequence after PCR amplification.

AB A method of detecting a specific nucleic acid sequence at very low levels using multiple hybridization probes labeled with a fluorescent reporter group is described. The target is hybridized with a mixt. of labeled probes and excess probe is removed after hybridization, particularly electrophoretically, esp. using a very high frequency a.c. (.gtoreq.100 kHz). This generates an electrophoretic trap that rapidly removes the probes from the origin, leaving hybridized probes and the target sequence near the origin where they can be quantified by **fluorescence correlation spectroscopy**. Peptide nucleic acids carrying a neutralizing charge may be used as probes. This lowers the mobility of the hybrid even farther compared to the probes themselves. The method is intended for use on very small (femtoliter) vols. and can be used to detect the amplification products of single copies of a sequence after PCR amplification.

IT **Nucleotides**

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses) (oligo-, probes, fluorescence-labeled; detection of nucleic acid sequences at very low concns. using multiple probes with fluorescent labels)

=>

file copy

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2000 ACS

AN 1998:458128 CAPLUS

DN 129:240429

TI Quantitation of changes in P0 mRNA by polymerase chain reaction in primary

cultured Schwann cells stimulated by axolemma-enriched fraction

AU Clive, Diana R.; Lopez, T. J.; DeVries, George H.

CS Research Service, Edward Hines Jr Department of Veterans Affairs Hospital, Hines, IL, 60141, USA

SO J. Neurosci. Methods (1998), 81(1,2), 25-34

CODEN: JNMEDT; ISSN: 0165-0270

PB Elsevier Science B.V.

DT Journal

LA English

AB A requirement for large nos. of primary culture cells has frequently restricted investigations of gene expression in glial cells. The authors have developed a non-radioactive method based on reverse transcription-polymerase chain reaction (RT-PCR) to accurately assess small changes in the expression of the myelin specific gene P0 in Schwann cells. Using axolemma-enriched fraction (AEF) as an inducing agent, the authors demonstrate that RT-PCR can be used to detect 4-8-fold increases in P0 mRNA levels occurring in a time and dose dependent manner,

utilizing only 250000 cells per assay. Initial expts. used an in vitro transcribed RNA for P0 constructed with a 300 bp deletion for quantitation by competitive RT-PCR. Relative quantitation by co-amplification of the housekeeping gene glyceraldehyde-phosphate dehydrogenase was established and provided similar results. Product evaluation was enhanced

50-100-fold by the incorporation of primers labeled with biotin at the 5' end, allowing for the sensitive detection of PCR product by enhanced chemiluminescence and autoradiog. This technique provides sensitivity to detect and evaluate picogram amts. of DNA. Our results validate the

assay for P0 gene expression and indicate that the technique should facilitate the study of multiple genes of interest in glial cell systems.

IT 213020-50-9D, 5'-biotin labeled

RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(nucleotide sequence of PCR primer P0 #1;

quantitation of changes in P0 mRNA by polymerase chain reaction in primary cultured Schwann cells stimulated by axolemma-enriched fraction)

IT 213020-55-4D, 5'-biotin labeled

RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(nucleotide sequence of PCR primer P0 #2;

quantitation of changes in P0 mRNA by polymerase chain reaction in primary cultured Schwann cells stimulated by axolemma-enriched fraction)

09/325,129

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Main Menu

Search Form

Posting Counts

Show S Numbers

Edit S Numbers

Preferences

Search Results -

Term	Documents
FLUORESCENCE.DWPI,EPAB,JPAB,USPT.	50412
FLUORESCENCES.DWPI,EPAB,JPAB,USPT.	339
CORRELATION.DWPI,EPAB,JPAB,USPT.	114992
CORRELATIONS.DWPI,EPAB,JPAB,USPT.	11579
SPECTROSCOP\$3	0
SPECTROSCOP.DWPI,EPAB,JPAB,USPT.	182
SPECTROSCOPA.DWPI,EPAB,JPAB,USPT.	2
SPECTROSCOPE.DWPI,EPAB,JPAB,USPT.	17557
SPECTROSCOPEA.DWPI,EPAB,JPAB,USPT.	1
SPECTROSCOPEC.DWPI,EPAB,JPAB,USPT.	1
(FLUORESCENCE CORRELATION SPECTROSCOP\$3 NEAR5 PCR).USPT,JPAB,EPAB,DWPI.	0

There are more results than shown above. [Click here to view the entire set.](#)

Database:	US Patents Full-Text Database	▲
	US Pre-Grant Publication Full-Text Database	
	JPO Abstracts Database	
	EPO Abstracts Database	
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	IBM Technical Disclosure Bulletins	▼

Refine Search:

fluorescence correlation spectroscop\$3
near5 PCR

Clear

Search History

Today's Date: 11/24/2001

<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
USPT,JPAB,EPAB,DWPI	fluorescence correlation spectroscop\$3 near5 PCR	0	<u>L5</u>
USPT,JPAB,EPAB,DWPI	fluorescence correlation spectroscop\$3 near5 nucleic acid amplif\$	0	<u>L4</u>
USPT,JPAB,EPAB,DWPI	12 and asymmetr\$2	3	<u>L3</u>
USPT,JPAB,EPAB,DWPI	11 and (nucleic acid amplification or PCR)	24	<u>L2</u>
USPT,JPAB,EPAB,DWPI	fluorescence correlation spectroscop\$3	72	<u>L1</u>

Term	Documents
NUCLEIC.DWPI,EPAB,JPAB,USPT.	72914
NUCLEICS.DWPI,EPAB,JPAB,USPT.	8
ACID.DWPI,EPAB,JPAB,USPT.	1655004
ACIDS.DWPI,EPAB,JPAB,USPT.	474490
AMPLIFICATION.DWPI,EPAB,JPAB,USPT.	135194
AMPLIFICATIONS.DWPI,EPAB,JPAB,USPT.	3245
PCR.DWPI,EPAB,JPAB,USPT.	27615
PCRS.DWPI,EPAB,JPAB,USPT.	771
(1 AND (PCR OR ((NUCLEIC ADJ ACID) ADJ AMPLIFICATION))).USPT,JPAB,EPAB,DWPI.	24

Documents, starting with Document:

Display Format:

WEST

Generate Collection

Search Results - Record(s) 1 through 10 of 24 returned.☐ 1. Document ID: US 6310687 B1

L2: Entry 1 of 24

File: USPT

Oct 30, 2001

US-PAT-NO: 6310687

DOCUMENT-IDENTIFIER: US 6310687 B1

TITLE: Light detection device with means for tracking sample sites

DATE-ISSUED: October 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stumbo; David P.	Belmont	CA		
Modlin; Douglas N.	Palo Alto	CA		

US-CL-CURRENT: 356/317; 250/458.1, 356/417, 422/63, 422/82.08

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 2. Document ID: US 6307037 B1

L2: Entry 2 of 24

File: USPT

Oct 23, 2001

US-PAT-NO: 6307037

DOCUMENT-IDENTIFIER: US 6307037 B1

TITLE: Fungal target genes and methods

DATE-ISSUED: October 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gaffney; Thomas Deane	Chapel Hill	NC		
Flavier; Albert	Raleigh	NC		
Cloyd Kirksey; Michelle M.	Durham	NC		
Phillippsen; Peter	Riehen			CHX
Dietrich; Frederick	Durham	NC		
Wendland; Jurgen	Lorrach			DEX
Bernasconi; Paul	Chapel Hill	NC		
White; Kimberly	Durham	NC		
Filipowicz; Witold	Riehen			CHX

US-CL-CURRENT: 536/23.1; 435/252.3, 435/254.11, 435/254.2, 435/320.1, 536/24.1

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 3. Document ID: US 6306607 B1

L2: Entry 3 of 24

File: USPT

Oct 23, 2001

US-PAT-NO: 6306607

DOCUMENT-IDENTIFIER: US 6306607 B1

TITLE: Heterogeneous assay for pyrophosphate

DATE-ISSUED: October 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; John G. K.	Lincoln	NE		

US-CL-CURRENT: 435/6; 436/501

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 4. Document ID: US 6300091 B1

L2: Entry 4 of 24

File: USPT

Oct 9, 2001

US-PAT-NO: 6300091

DOCUMENT-IDENTIFIER: US 6300091 B1

TITLE: Herbicide target genes and methods

DATE-ISSUED: October 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Patton; David A.	Durham	NC		
Ashby; Carl S.	Boulder	CO		
McElver; John A.	Durham	NC		

US-CL-CURRENT: 435/15; 435/193

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 5. Document ID: US 6297018 B1

L2: Entry 5 of 24

File: USPT

Oct 2, 2001

US-PAT-NO: 6297018
DOCUMENT-IDENTIFIER: US 6297018 B1

TITLE: Methods and apparatus for detecting nucleic acid polymorphisms

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
French; Todd E.	Cupertino	CA		
Modlin; Douglas N.	Palo Alto	CA		
Owicki; John C.	Palo Alto	CA		
Richey; James S.	Palo Alto	CA		
Leytes; Lev J.	Palo Alto	CA		
Razvi; Enal S.	San Francisco	CA		

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/24.3

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMMC	Draw Desc	Image
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☐ 6. Document ID: US 6294345 B1

L2: Entry 6 of 24

File: USPT

Sep 25, 2001

US-PAT-NO: 6294345
DOCUMENT-IDENTIFIER: US 6294345 B1

TITLE: Genes encoding proteins essential for plant growth and methods of use

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Levin; Joshua Zvi	Durham	NC		
Bauer; Michael William	Holly Springs	NC		
Zheng; Feng	West Lafayette	IN		

US-CL-CURRENT: 435/7.1; 435/18, 435/195, 435/232, 530/370

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMMC	Draw Desc	Image
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☐ 7. Document ID: US 6291665 B1

L2: Entry 7 of 24

File: USPT

Sep 18, 2001

US-PAT-NO: 6291665
DOCUMENT-IDENTIFIER: US 6291665 B1
TITLE: Fungal target genes and methods
DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gaffney; Thomas Deane	Chapel Hill	NC		
Flavier; Albert	Raleigh	NC		
Gates; Krista	Apex	NC		
Wendland; Jurgen	Jena			DEX
Ayad-Durieux; Yasmina	Basel			CHX
Dietrich; Fred	Basel			CHX
Philippsen; Peter	Riehen			CHX

US-CL-CURRENT: 536/23.74; 435/252.3, 435/254.11, 435/320.1

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 8. Document ID: US 6291660 B1

L2: Entry 8 of 24

File: USPT

Sep 18, 2001

US-PAT-NO: 6291660
DOCUMENT-IDENTIFIER: US 6291660 B1

TITLE: Fungal genes required for normal growth and development

DATE-ISSUED: September 18, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gaffney; Thomas Deane	Chapel Hill	NC		
Wendland; Juergen	Lorrach			DEX
Dietrich; Fred	Basel			CHX
Philippsen; Peter	Riehen			CHX
Goff; Stephen Arthur	Encinitas	CA		

US-CL-CURRENT: 536/23.1

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 9. Document ID: US 6263286 B1

L2: Entry 9 of 24

File: USPT

Jul 17, 2001

US-PAT-NO: 6263286
DOCUMENT-IDENTIFIER: US 6263286 B1

TITLE: Methods of analyzing polymers using a spatial network of fluorophores and fluorescence resonance energy transfer

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gilmanshin; Rudolf	Waltham	MA		
Chan; Eugene Y.	Boston	MA		

US-CL-CURRENT: 702/19; 435/6

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 10. Document ID: US 6258533 B1

L2: Entry 10 of 24

File: USPT

Jul 10, 2001

US-PAT-NO: 6258533

DOCUMENT-IDENTIFIER: US 6258533 B1

TITLE: Iterative and regenerative DNA sequencing method

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; Douglas H.	Iowa City	IA		

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Generate Collection

WEST

Generate Collection

Search Results - Record(s) 11 through 21 of 24 returned.

☐ 11. Document ID: US 6245506 B1

L2: Entry 11 of 24

File: USPT

Jun 12, 2001

US-PAT-NO: 6245506

DOCUMENT-IDENTIFIER: US 6245506 B1

TITLE: Integrated sequencing device

DATE-ISSUED: June 12, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laugharn, Jr.; James A.	Winchester	MA		
Hess; Robert A.	Cambridge	MA		

US-CL-CURRENT: 435/6; 250/282, 422/68.1, 536/23.1, 536/25.3, 549/22

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 12. Document ID: US 6242188 B1

L2: Entry 12 of 24

File: USPT

Jun 5, 2001

US-PAT-NO: 6242188

DOCUMENT-IDENTIFIER: US 6242188 B1

TITLE: Sample processing to release nucleic acids for direct detection

DATE-ISSUED: June 5, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dattagupta; Nanibhushan	San Diego	CA		
Sridhar; C. Nagaraja	San Diego	CA		
Wu; Whei-Kuo	San Diego	CA		

US-CL-CURRENT: 435/6; 435/243, 435/259, 435/91.1, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMOC	Draw Desc	Image
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☐ 13. Document ID: US 6232075 B1

L2: Entry 13 of 24

File: USPT

May 15, 2001

US-PAT-NO: 6232075

DOCUMENT-IDENTIFIER: US 6232075 B1

TITLE: Heterogeneous assay for pyrophosphate detection

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Williams; John G. K.	Lincoln	NE		

US-CL-CURRENT: 435/6; 436/501, 536/24.3, 536/24.31

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 14. Document ID: US 6221660 B1

L2: Entry 14 of 24

File: USPT

Apr 24, 2001

US-PAT-NO: 6221660

DOCUMENT-IDENTIFIER: US 6221660 B1

TITLE: DNA encoding SNORF25 receptor

DATE-ISSUED: April 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bonini; James A.	Oakland	NJ		
Borowsky; Beth E.	Montclair	NJ		
Adham; Nika	Ridgewood	NJ		
Boyle; Noel	Cliffside Park	NJ		
Thompson; Thelma O.	Passaic Park	NJ		

US-CL-CURRENT: 435/348; 435/357, 435/361, 435/365, 435/369, 530/350, 536/23.5

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 15. Document ID: US 6201113 B1

L2: Entry 15 of 24

File: USPT

Mar 13, 2001

US-PAT-NO: 6201113
DOCUMENT-IDENTIFIER: US 6201113 B1
TITLE: Zymogenic nucleic acid molecules
DATE-ISSUED: March 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Todd; Alison V.	Glebe NSW		2037	AUX
Fuery; Caroline J.	Sydney NSW			AUX
Cairns; Murray J.	Woy Woy NSW		2256	AUX

US-CL-CURRENT: 536/23.2; 536/24.33

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KIMC	Draw Desc	Image
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☐ 16. Document ID: US 6190889 B1

L2: Entry 16 of 24 File: USPT Feb 20, 2001
US-PAT-NO: 6190889
DOCUMENT-IDENTIFIER: US 6190889 B1

TITLE: Methods for removing primer sequences and blocking restriction
endonuclease recognition domains

DATE-ISSUED: February 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; Douglas H.	Iowa City	IA		

US-CL-CURRENT: 435/91.1; 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KIMC	Draw Desc	Image
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☐ 17. Document ID: US 6187566 B1

L2: Entry 17 of 24 File: USPT Feb 13, 2001

US-PAT-NO: 6187566
DOCUMENT-IDENTIFIER: US 6187566 B1

TITLE: Method of labeling a nucleic acid amplicon with simultaneous contamination prevention

DATE-ISSUED: February 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dattagupta; Nanibhushan	San Diego	CA		
Sridhar; C. Nagaraja	San Diego	CA		
Wu; Whei-Kuo	San Diego	CA		

US-CL-CURRENT: 435/91.1; 435/18, 435/4, 435/6, 436/501, 436/504, 436/63, 436/94,
536/25.32

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 18. Document ID: US 6140055 A

L2: Entry 18 of 24

File: USPT

Oct 31, 2000

US-PAT-NO: 6140055
DOCUMENT-IDENTIFIER: US 6140055 A

TITLE: Zymogenic nucleic acid detection methods and related kits

DATE-ISSUED: October 31, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Todd; Alison V.	Glebe			AUX
Fuery; Caroline J.	Sydney			AUX
Cairns; Murray J.	Woy Woy			AUX

US-CL-CURRENT: 435/6; 435/91.2, 536/23.2, 536/24.33

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 19. Document ID: US 6117990 A

L2: Entry 19 of 24

File: USPT

Sep 12, 2000

US-PAT-NO: 6117990
DOCUMENT-IDENTIFIER: US 6117990 A
TITLE: DNA encoding SNORF1 receptor
DATE-ISSUED: September 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bonini; James A.	Oakland	NJ		
Borowsky; Beth E.	Montclair	NJ		

US-CL-CURRENT: 536/23.5; 536/23.1, 536/24.3

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KIMC	Draw Desc	Image
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☐ 20. Document ID: US 6110676 A

L2: Entry 20 of 24

File: USPT

Aug 29, 2000

US-PAT-NO: 6110676
DOCUMENT-IDENTIFIER: US 6110676 A

TITLE: Methods for suppressing the binding of detectable probes to non-target sequences in hybridization assays

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Coull; James M.	Westford	MA		
Hyldig-Nielsen; Jens J.	Holliston	MA		
Godtfredsen; Sven E.	V.ae butted.rl.o slashed.se			DKX
Fiandaca; Mark J.	Acton	MA		
Stefano; Kyriaki	Hopkinton	MA		

US-CL-CURRENT: 435/6; 435/5, 435/7.1, 435/91.1, 435/91.2, 530/300, 530/350, 530/387.1, 530/388.1, 536/23.1, 536/24.3, 536/24.31, 536/24.32, 536/24.33

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KIMC	Draw Desc	Image
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☐ 21. Document ID: US 5858671 A

L2: Entry 21 of 24

File: USPT

Jan 12, 1999

US-PAT-NO: 5858671

DOCUMENT-IDENTIFIER: US 5858671 A

TITLE: Iterative and regenerative DNA sequencing method

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; Douglas H.	Iowa City	IA		

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMCC	Draw Desc	Image
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[Generate Collection](#)

Term	Documents
NUCLEIC.DWPI,EPAB,JPAB,USPT.	72914
NUCLEICS.DWPI,EPAB,JPAB,USPT.	8
ACID.DWPI,EPAB,JPAB,USPT.	1655004
ACIDS.DWPI,EPAB,JPAB,USPT.	474490
AMPLIFICATION.DWPI,EPAB,JPAB,USPT.	135194
AMPLIFICATIONS.DWPI,EPAB,JPAB,USPT.	3245
PCR.DWPI,EPAB,JPAB,USPT.	27615
PCRS.DWPI,EPAB,JPAB,USPT.	771
(1 AND (PCR OR ((NUCLEIC ADJ ACID) ADJ AMPLIFICATION))).USPT,JPAB,EPAB,DWPI.	24

[Display](#)[22](#)

Documents, starting with Document:

[22](#)**Display Format:**[CIT](#)[Change Format](#)

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Search Results - Record(s) 22 through 24 of 24 returned.☐ 22. Document ID: US 5807677 A

L2: Entry 22 of 24

File: USPT

Sep 15, 1998

US-PAT-NO: 5807677

DOCUMENT-IDENTIFIER: US 5807677 A

TITLE: Method for direct identification of few nucleic acid strands

DATE-ISSUED: September 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eigen; Manfred	Gottingen			DEX
Rigler; Rudolf	Danderyd			SEX

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2, 536/22.1, 536/23.1, 536/24.3, 536/25.3

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 23. Document ID: US 5674743 A

L2: Entry 23 of 24

File: USPT

Oct 7, 1997

US-PAT-NO: 5674743

DOCUMENT-IDENTIFIER: US 5674743 A

TITLE: Methods and apparatus for DNA sequencing

DATE-ISSUED: October 7, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ulmer; Kevin M.	Cohasset	MA		

US-CL-CURRENT: 435/287.2; 422/82.08, 435/288.7, 436/172, 436/94

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 24. Document ID: DE 19950823 A1, JP 2000125900 A

L2: Entry 24 of 24

File: DWPI

Apr 27, 2000

DERWENT-ACC-NO: 2000-367174
DERWENT-WEEK: 200032
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TITLE: Quantitative determination of a target nucleic acid in a biological sample comprises amplification using a known number of labeled primer molecules

INVENTOR: KINJO, M

PRIORITY-DATA: 1998JP-0301316 (October 22, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 19950823 A1	April 27, 2000		017	C12Q001/68
JP 2000125900 A	May 9, 2000		012	C12Q001/68

INT-CL (IPC): C12N 15/09; C12Q 1/68; G01N 33/50

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Clip Img	Image
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Term	Documents
NUCLEIC.DWPI,EPAB,JPAB,USPT.	72914
NUCLEICS.DWPI,EPAB,JPAB,USPT.	8
ACID.DWPI,EPAB,JPAB,USPT.	1655004
ACIDS.DWPI,EPAB,JPAB,USPT.	474490
AMPLIFICATION.DWPI,EPAB,JPAB,USPT.	135194
AMPLIFICATIONS.DWPI,EPAB,JPAB,USPT.	3245
PCR.DWPI,EPAB,JPAB,USPT.	27615
PCRS.DWPI,EPAB,JPAB,USPT.	771
(1 AND (PCR OR ((NUCLEIC ADJ ACID) ADJ AMPLIFICATION))).USPT,JPAB,EPAB,DWPI.	24

Display

22

Documents, starting with Document:

24

Display Format:

CIT

Change Format

WEST

Generate Collection

L2: Entry 22 of 24

File: USPT

Sep 15, 1998

DOCUMENT-IDENTIFIER: US 5807677 A

TITLE: Method for direct identification of few nucleic acid strands

BSPR:

One method which was developed a number of years ago involves amplifying the individual or few nucleic acid strands to a concentration level suitable for conventional methods such as gel electrophoresis, the process of temperature gradient gels, sequencing, for example according to the Sanger Method or the Maxam-Gilbert technique. Polymerase chain reactions (PCR) or analog methods are available for this amplification. These are based on a specific replication of the nucleic acid sections to be identified. One hereby exploits the properties of DNA-polymerases, which can polymerise a single strand to a double-strand if they are provided with a short, double-stranded section as a primer. The nucleic acid strands containing a sequence to be amplified are mixed with two chemically synthesized oligonucleotides originating from the end areas of the sequence to be amplified and which are strand-specific, i.e. complementary to one of the two DNA strands.

BSPR:

On the one hand such methods are very time-consuming; not only do the PCR reactions have to be carried out but these are then followed by a number of further analyses so as to be able to at least quantitatively conclude the presence of the desired sequence in the test solution. Moreover, certain short-chain nucleic acid sequences with a length of approx. 15-20 nucleotides have to be provided which are exactly complementary to a corresponding section of the sequence to be detected. Such an exactly complementary short-chain nucleic acid section is generally called an "antisense sequence" or "primer".

BSPR:

Apart from the time involved, this method also has the disadvantage that only those nucleic acid sequences can be identified where at least a short section of the sequential sequence at both ends is known so that the corresponding primer can be provided. If the nucleic acid strands to be identified are RNA molecules, these first have to be transcribed into a DNA copy by means of an RNA-dependent DNA polymerase before the PCR method can be employed.

BSPR:

A further method consists of directly visualizing the sequence to be identified, which means that naturally this sequence first has to be clearly distinguishable from alternative sequences. A newly developed method to identify and count individual molecules is the fluorescence correlation spectroscopy described in WO 94/16313, hereinafter referred to as the FCS method. The publication quoted explains how single DNA/RNA molecules marked with dye can be identified by FCS in a very small probe volume in the range of 0.1-10 fl provided the molecule to be identified either differs significantly from alternative molecules in the probe volume or has been isolated.

BSPR:

In the PCR method already mentioned above, two primer sequences are required of which one is complementary to the start region of the plus strand and the other complementary to the start region of the minus strand. The sequence section between the positions of the two primer sequences is then amplified. With the FCS method all that is required is a specific primer sequence marked with a

fluorescence dye. This hybridizes directly on to (single-stranded) RNA sequence to be detected or to a strand of the melted DNA sequence. In this way the marked primers differ greatly from the free primers in both their charge characteristics and their mobility and can be directly detected by FCS without further amplification.

BSPR:

The FCS method thus has a number of advantages compared to the PCR method or analog methods. Firstly, the FCS method can be used directly for both single-stranded and double-stranded nucleic acid molecules. Since a number of pathogens are single-stranded RNA viruses, with the FCS method these do not firstly have to be transcribed to DNA, which has a positive effect on the time required for the analysis. Moreover, the FCS method is more direct, identifying the individual nucleic acids which otherwise first would have to be amplified when using the PCR method. This also leads to a noticeable difference in the time required.

BSPR:

In addition, the amplification process can be disturbed or hindered by certain substances so that the PCR method is not always reliable for natural samples.

BSPR:

However, just like the PCR method, the FCS method is susceptible to faults with respect to its specificity. Partial sections of a primer sequence, e.g., may also be complementary to sequences which are by chance similar to the target sequence. Primer sequences of lengths between 15 and 20 nucleotides are thus selected to achieve an adequate specificity of binding. However, it then has further to be ensured that the melting points of only partially complementary sequences are below the working temperature, in other words cannot be measured.

BSPR:

In the PCR method two primer sequences are used, whereby the probability that both sequences display faulty bindings is slight. However, the primers have to be used in much higher concentrations since they are consumed by the reaction.

BSPR:

These are well established methods which can be employed advantageously to produce the primer from the target sequence. In this manner primer can be advantageously produced against target sequences whose sequence succession is only partly known or completely unknown. All that is necessary is that the ends of the target sequences are known or that known sequence sections are synthesized to completely unknown sequence sections so that the primers necessary for the PCR method can be provided. The section of the target sequence between the two primers does not have to be known in the succession, the correct primers for the analysis are "automatically" produced, as it were, so that new viruses can also be looked for.

CLPR:

9. A method in accordance with claim 7, wherein the replication or transcription product is produced by a method selected from the group consisting of the polymerase chain reaction technique (PCR technique), an PCR analog method and other methods utilizing specific RNA or DNA polymerases, and combinations thereof.

CLPR:

16. A method in accordance with claim 1, wherein the identification of the target sequence is carried out by a fluorescence correlation spectroscopy whereby a small volume element of the incubated solution, is exposed to an excitation light from a laser which excites the primers in this probe volume to emit fluorescence light, the fluorescence light emitted from the probe volume is measured with a photo-detector and a correlation is drawn between the temporal change in the measured emission and the relative rate of diffusion of the molecules involved, so that with a correspondingly stronger dilution, individual molecules can be identified in the probe volume.

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DEPR:

If it is desired to amplify any of the isolated DNA or a specific portion thereof, prior to sequencing, polymerase chain reaction (PCR) can be employed (U.S. Pat. Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc. Natl. Acad. Sci. USA 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220, which are incorporated herein by reference).

ORPL:

Rigler and Widengren, 1990, "Ultrasensitive detection of single molecules by fluorescence correlation spectroscopy," in BioScience, B. Klinge & C. Owmar (eds.), pp. 180-183.